S4.P1

Isolation and identification of mitochondrial complex I subunits by three-dimensional electrophoresis: Applications

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Mitochondrial complex I or NADH:ubiquinone oxidoreductase catalyses the oxidation of matrix NADH by membrane ubiquinone and is a major entry point for electrons into the respiratory chain. It is the biggest complex of the mitochondrial respiratory chain, composed of at least 44 subunits with a molecular mass of about 1 MDa [1;2]. 14 subunits found in the bacterial enzyme represent the minimal catalytic core unit providing the catalytic activity of the enzyme while the function of the 30 remaining so-called accessory subunits found in the mammalian enzyme is still under debate. The study of these accessory subunits represents a challenge in the understanding of the mammalian complex I functioning, regulation and assembly. Analysing 44 subunits at the same time is not trivial, therefore we specifically implement three-dimensional electrophoresis to study the organisation of the enzyme. Here, we present a quick and reliable method for complex I subunit separation, and a reference "map" of the distribution of all complex I subunits after separation by BN/dSDS-PAGE without the commonly used electroelution step to extract protein from a BN-PAGE. The obtained subunit distribution from complex I isolated by BN-PAGE matches the distribution of the subunits of purified bovine complex I [3]. This map was further used to gain information on the accessibility of the mammalian complex I subunits using two different approaches. The intact, fully active enzyme was labelled with amino acid specific fluorescent probes or submitted to a limited proteolysis. The most accessible subunits presumably located at the surface of complex I (such as B8, B18 and AQDQ) were found. This map can now be used to quickly identify every subunit for numerous applications, including enzyme assembly studies, DIGE-like approach or cross-linking.

References

[1] J. Carroll, I.M. Fearnley, J.M. Skehel, R.J. Shannon, J. Hirst, J.E. Walker, Bovine complex I is a complex of 45 different subunits, J. Biol. Chem. 281 (2006), 32724–32727.
[2]. E. Balsa, R. Marco, E. Perales-Clemente, R. Szklarczyk, E. Calvo, M.O. Landázuri, J.A., Enríquez, J. A., NDUFA4 is a subunit of complex IV of the mammalian electron transport chain, Cell Metab.16 (2012), 378–386.
[3] M.S. Sharpley, R.J. Shannon, F. Draghi, J. Hirst, Interactions between phospholipids and NADH:ubiquinone oxidoreductase (complex I) from bovine mitochondria, Biochemistry 45 (2006), 241–248.

doi:10.1016/j.bbabio.2014.05.049

S4.P2

The role of proton and sodium ions on the bioenergetics of *Escherichia coli*. The involvement of respiratory complex I

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Respiratory complex I plays a central role in energy transduction. It catalyzes the oxidation of NADH and the reduction of quinone coupled to ion translocation across the membrane, thereby establishing an electrochemical potential. Its crystallographic structure has been resolved [1], but still, the mechanisms of quinone reduction, ion translocation and their coupling are unknown. The nature of the ion(s) translocated by this enzyme is still a highly discussed issue, being H^+ and Na^+ possible candidates. It is generally accepted that H^+ is the coupling ion in complex I [2], however in the case of some bacterial complexes I Na^+ has been proposed to have that role [3]. It was also shown that some bacterial complexes I are capable of H^+ and Na^+ translocation, but in opposite directions, with H^+ being the coupling ion [4]. In the present work, we aim to address the role of the two different ions, H^+ and Na^+, in the overall cellular bioenergetics of the bacterium *Escherichia coli*. Particularly, we study the influence of pH and sodium ions on the growth of *E. coli* KNabc (a strain mutated in all known Na^+/H^+ antiporters) and on *E. coli* KNabc complemented with the *nhaA* gene (a gene that codifies for NhaA). We also investigate the influence of proton and sodium ions on the redox activity of *E. coli* respiratory complex I, as well as, on the ion transport process. NADH:quinone oxidoreductase activity was determined at different pH values (6.8, 7.5 and 8.2) and at different sodium concentrations (0–250 mM). Transport assays in the same conditions were also performed. Implications on the coupling mechanism of complex I are discussed.

References

[1] R.G. Efremov, R. Baradaran, L.A. Sazanov, The architecture of respiratory complex I, Nature 465 (2010) 441–445.
[2] A. Galkin, S. Drose, U. Brandt, The proton pumping stoichiometry of purified mitochondrial complex I reconstituted into proteoliposomes, Biochimica et biophysica acta 1757 (2006) 1575–1581.
[3] J. Steuber, C. Schmid, M. Rufibach, P. Dimroth, Na^+ translocation by complex I (NADH:quinone oxidoreductase) of *Escherichia coli*, Molecular microbiology 35 (2000) 428–434.
[4] A.P. Batista, M.M. Pereira, Sodium influence on energy transduction by complexes I from *Escherichia coli* and *Paracoccus denitrificans*, Biochimica et biophysica acta 1807 (2011) 286–292.

doi:10.1016/j.bbabio.2014.05.050

S4.P3

Mutagenesis of complex I from *Thermus thermophilus*

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NADH:ubiquinone oxidoreductase (complex I) is situated on the inner mitochondrial membrane, or the plasma membrane of most bacteria. Complex I couples the transfer of two electrons from NADH to ubiquinone with the translocation of four protons across a membrane [1] and is the first complex in the electron transport